

Attached is the report of the in-house investigations of Papain in the
developing chicken embryo. 11-4-75

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MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

TO : GRAS Review Branch, HFF-335

DATE: November 4, 1975

Dr. Herbert Blumenthal, Acting Director
Division of Toxicology, HFF-150

FROM : M. Jacqueline Verrett, Ph.D. *M. Jacqueline Verrett*
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SUBJECT: Investigation of the Toxic and Teratogenic Effects of GRAS Substance to
the Developing Chicken Embryo.

Attached is the report of the in-house investigations of Papain in the
developing chicken embryo.

Investigations of the Toxic and Teratogenic Effects of
GRAS Substances to the Developing Chicken
Embryo: Papain

Protocol:

Papain (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in water as the solvent by two routes and at two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2,3).

Groups of fifteen or more eggs were treated under these four conditions at several dose levels until a total of seventy-five to one hundred eggs per level was reached for all levels allowing some hatch. Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in tables 1 through 4 for each of the four conditions of test.

Columns 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i.e., total non-viable divided by total treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia or other nerve disorders.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Columns 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and the untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data of columns 4, 5 and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

Discussion:

There was slight toxicity above the solvent controls for all conditions of test but the regression of mortality on dose was significant only for yolk treatment at 96 hours, with a calculated LD₅₀ of 2.3981 mg/kg (0.1199 mg/egg).

Scattered minor abnormalities were observed for all four conditions of test, but in no instance were these significantly higher in incidence or different in nature from those observed in the solvent-treated or untreated control eggs. Papain displayed no teratogenicity under the test conditions employed.

1. Papain, Miles Laboratories, Elkhart, Indiana. Lot No. C-4622
2. McLaughlin, J., Jr., Marliac, J. P., Verrett, M. Jacqueline, Matchler, Mary K., and Fitzhugh, O. G., (1963) Toxicol. Appl. Pharmacol. 5, 760-770
3. Verrett, M. J., Marliac, J. P., and McLaughlin, J., Jr., (1964) JAOAC 47, 1002 - 1006
4. Finney, D. J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendix I.

Papain
Air Cell @ 0 Hours

Table 1

Dose		Number of Eggs	**Percent Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
10.00	200.00	115	39.13*	1.73	0.86
5.00	100.00	114	36.84*	0.87	0.00
2.50	50.00	115	35.65*	0.00	0.00
1.250	25.00	113	38.05*	3.53	0.88
0.500	10.00	115	33.04*	4.34	0.86
Water	--	149	21.47	0.67	0.67
Controls	--	628	12.42	1.27	1.11

**Slope is negative

*Significantly different from solvent p \leq 0.05

Papain
Air Cell @ 96 Hours

Table 2

Dose		Number of Eggs	**Percent Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
5.00	100.00	119	36.97*	2.52	0.84
2.50	50.00	125	45.60*	0.00	0.00
1.250	25.00	125	33.80*	0.80	0.80
0.6250	12.50	125	36.80*	1.60	0.80
0.250	5.00	124	37.09*	1.61	1.61
Water	--	145	13.10	1.37	0.68
Controls	--	628	12.42	1.27	1.11

**Slope not significantly different from zero $p = 0.05$

*Significantly different from solvent $p \leq 0.05$

Papain
Yolk @ 0 Hours

Table 3

Dose		Number of Eggs	**Percent Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
10.00	200.00	120	80.00*	0.00	0.00
5.00	100.00	120	80.83*	0.00	0.00
2.50	50.00	120	79.16*	0.00	0.00
1.250	25.00	120	81.66*	0.00	0.00
0.500	10.00	120	75.83*	0.00	0.00
Water	--	160	43.12	0.00	0.00
Controls	--	628	12.42	1.27	1.11

**Slope not significantly different from zero $p = 0.05$

*Significantly different from solvent $p \leq 0.05$

Papain
Yolk @ 96 Hours

Table 4

Dose		Number of Eggs	**Percent Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
5.00	100.00	105	91.42*	0.00	0.00
2.50	50.00	105	89.52*	3.80	0.95
1.250	25.00	105	93.33*	0.95	0.95
0.6250	12.50	105	78.09*	2.85	0.95
0.250	5.00	105	71.42*	3.80	0.95
Water	--	140	31.42	4.28	2.85
Controls	--	628	12.42	1.27	1.11

**LD₅₀ 2.3981 mg/kg (0.1199 mg/egg)

* Significantly different from solvent $p \leq 0.05$